Long-Term Lime Pretreatment of Poplar Wood

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Long-term lime pretreatment has proven to increase digestibility of many herbaceous lignocellulose sources; but until this work, its effects had not been evaluated on wood, whose lignin content is higher, and therefore, more recalcitrant to enzymatic hydrolysis. In this study, the mild conditions of long-term lime pretreatment (1-atm pressure, temperatures ranging from 25 to 75°C, and reaction times between 1 and 12 weeks, with and without air) were systematically applied to poplar wood available in two batches with different lignin contents. These batches were designated as low-lignin biomass (LLB) with lignin content of 21.4% and high-lignin biomass (HLB) with lignin content of 29.1%. Full factorial designs resulted in 79 samples of pretreated poplar that were analyzed for lignin and carbohydrates pretreatment yields, and enzymatic digestibility (15 FPU/g glucan in raw biomass cellulose loading). After aerated lime pretreatment at 65°C for 4 weeks, and subsequent enzymatic hydrolysis, an overall yield of 0.76 g glucan + xylan recovered per gram glucan + xylan in raw biomass was obtained. This is equivalent to an increased poplar wood digestibility of 7.5-fold compared with untreated biomass. Different batches of the feedstock resulted in different lignin and carbohydrates pretreatment yields; however, overall yields of carbohydrates (combining pretreatment and enzymatic hydrolysis) were similar. © 2010 American Institute of Chemical Engineers *AIChE J,* 57: 1320–1328, 2011
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Introduction

Long-term lime pretreatment is initiated by mixing lignocellulosic biomass with excess calcium hydroxide (lime loaded as 0.5 g/g dry biomass) and water (9–15 g/g dry biomass). Then, for days to weeks, the mixture is exposed to temperatures ranging from 25 to 75°C at atmospheric pressure, preferably with aeration.[1] This procedure significantly increases lignocellulosic biomass digestibility making it useful as livestock feed or feedstock for fermentation processes to produce liquid fuels or chemicals.[2]

Chemically, the main results of alkaline pretreatments are cleavage of lignin polymer and hydrolysis of acetyl groups on hemicellulose; however, some undesirable carbohydrate degradation also occurs.[3,4] These outcomes result from reactions between hydroxyl groups and lignocellulose through mechanisms that strongly depend on temperature and time. Among alkalis, lime is preferred because it is safe to handle, inexpensive, easy to recover, compatible with oxidants, and results in good carbohydrate preservation.[5] Under alkaline conditions,

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lignin removal is significantly enhanced by oxidative agents.[6–8] Among these, air is preferred because it is inexpensive.

A previous study with herbaceous biomass showed that wheat straw (lignin content not reported) and sugarcane bagasse (22% lignin) treated with lime without oxidant for 24 h at 65°C increased digestibility 3–4 times compared with raw biomass.[3] In another study, sugarcane bagasse was lime treated with air for 4 weeks at 57°C, which resulted in an increased sugar yield of about five times compared with raw biomass.[9] Corn stover (18% lignin) treated with lime and air for 4 weeks at 55°C gave overall yields of glucose and xylose of 93.2 and 79.5%, respectively.[4]

Compared with herbaceous biomass, wood is more recalcitrant because of higher lignin content[10]; however, even in this case, alkali pretreatment increases biomass digestibility. For example, aspen soaked in NaOH solution at room temperature for 1 h increased digestibility (32%) with similar results for black ash (17%) and soft maple (20%).[11]

This work assesses long-term lime pretreatment of poplar wood on the basis of sugar yields using two different batches with different lignin contents (21.4 and 29.1%). The resulting mass balances, pretreatment yields, and overall yields are reported after pretreatment, enzymatic hydrolysis, and the combined pretreatment and enzymatic hydrolysis. This article statistically compares pretreatment yields, enzymatic digestibility of pretreated samples against untreated samples, the two biomass batches, and the effects of the presence or not of air during pretreatment.

In a previous study, lime pretreatment was successfully applied to poplar wood (HLB) at temperatures ranging between 110 and 180°C, oxygen pressure ranging between 14.8 and 28.1 bar, and pretreatment times between 1 and 6 h.[12] This type of lime pretreatment (designated as short-term pretreatment) is compared with long-term pretreatment for poplar wood elsewhere (Sierra et al., unpublished data).

Materials and Methods

Feedstock

Hybrid poplar wood (var NM6, genotype *P. nigra* × *P. maximowiczii*), was kindly provided by the National Renewable Energy Laboratory (NREL) in two batches. The procedure to prepare the poplar wood and reduce its particle size is explained elsewhere.[12] For low-lignin biomass (LLB), the composition of raw poplar wood determined by NREL is 45.1% glucan, 17.8% xylan, 1.7% mannan, 21.4% lignin, 1.5% galactan, 0.6% arabinan, 3.4% extractives, 0.8% ash, and 5.6% acetyl. For high-lignin biomass (HLB), the composition is 43.8% glucan, 14.9% xylan, 3.9% mannan, 29.1% lignin, 1.3% galactan, 0.7% arabinan, 3.3% extractives, 1.1% ash, and 3.3% acetyl.

Pretreatment

Lime pretreatment was performed in packed-bed reactors made of PVC pipe (1-inch = 0.0254-m ID, 17 inch = 0.432-m length) jacketed with larger diameter PVC pipes (2-inch = 0.0508-m ID, 15-inch = 0.381-m length). The desired temperature was maintained by constantly pumping water (3/4-hp = 0.560-kW centrifugal pumps, TEEL, Niles, IL) through the jacket from tanks (8-gallon = 0.0302-m^3, Nagalene, Mickleton, NJ) equipped with heating elements, a temperature controller (Omega Engineering, Stamford, CT), and a liquid-level controller (McMaster-Carr, Atlanta, GA). Several temperatures and aeration conditions were run simultaneously by using 40 of these PVC reactors attached to a metal frame, four pumps, and four tanks (Figure 1). Once the pretreatment temperature was reached, biomass (15 g dry weight), excess lime (7.5 g calcium hydroxide, certified), and distilled water (150 g) were mixed and charged into the reactors, thereby occupying 79% of reactor total volume. pH of the samples obtained in this way ranged between 11 and 11.5.

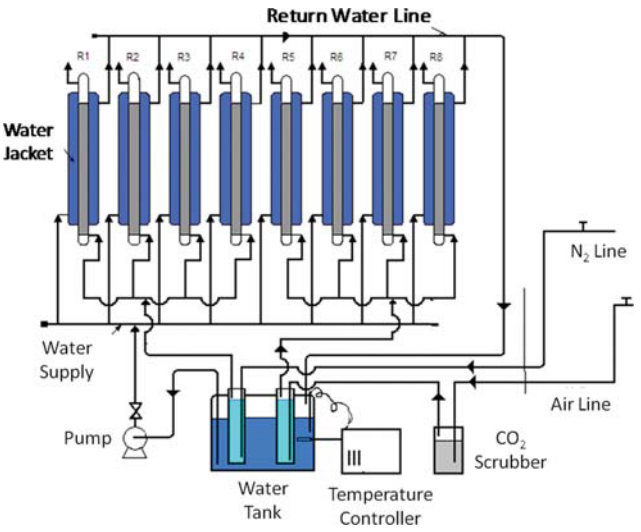

Figure 1. Schematic representation of a set of eight reactors used for pretreatment.

[Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

For some pretreatments, aeration was provided in gross excess using compressed air that was continuously bubbled into the reactors from the bottom at a flow rate of about 3.5 mL/min (air residence time 62 min and air superficial velocity 6.91 mm/min). This air flow rate was controlled by clamps located at the inlet. Before entering the reactors, the air was scrubbed of carbon dioxide by passing through a mixture of lime and water to limit neutralization of the lime in the reactors by the carbon dioxide in the air. After this, air was preheated and humidified by passing through a cylinder containing water at the pretreatment temperature.

Aeration was then started by bubbling compressed air into reactors using valves and clamps to control flow rate. To stop pretreatment, the air valves were closed, the pumps were turned off, the reactors were removed from the metal frame, and their content was carefully and completely transferred to properly labeled 1-L centrifuge bottles using ~500 mL of clear distilled water. pH of this mixture ranged between 10 and 11.

Lime consumption and biomass conditioning

To calculate unreacted lime, the biomass and liquid mixture obtained after pretreatment were carefully titrated using 5-N HCl. During pretreatment, lime was consumed in the following reactions: (1) neutralization of acetic acid (coming from acetyl in biomass), (2) neutralization of minerals in

Table 1. Pretreatment and Overall Yields for HLB and LLB at 75°C

Time (weeks)	Pretreatment yields*						Overall carbohydrates	
	Lignin		Glucan		Xylan		Combined yields†	
	HLB	LLB	HLB	LLB	HLB	LLB	HLB	LLB
0	1.00	1.00	1.00	1.00	1.00	1.00	0.10	0.09
1	0.76	0.75	0.93	0.87	0.98	0.80	0.33	0.41
2	0.61	0.65	0.93	0.85	0.85	0.65	0.41	0.50
4	0.50	0.56	0.79	0.69	0.74	0.55	0.58	0.49
8	0.31	0.35	0.65	0.48	0.69	0.34	0.55	0.46

*g component per gram component in raw biomass.
†g glucan + xylan per gram glucan + xylan in raw biomass.

ash, and (3) reactions that degrade/solubilize lignin and carbohydrates.

Lime consumed in reactions (1) and (2) was low and uniform for all pretreated samples because ash and acetyl contents are low (see Section Feedstock). Furthermore, acetic acid in pretreated samples was <0.01% regardless pretreatment conditions (measured using NREL Standard Analytical Procedure[13]); thus, lime consumption on reactions (1) and (2) was low and uniform for all samples. Consequently, differences in lime consumption for different pretreatment conditions are always due to reactions between the highly alkaline-oxidizing reaction media and lignin and carbohydrates.

After neutralization, the samples were extensively washed with deionized water and filtered using a vacuum filtration apparatus with Whatman 934/AH glass fiber filter paper (particle retention = 1.5 μm, Fisher Scientific, Pittsburgh, PA). Subsequently, the biomass was air dried. The weight of dried biomass and its moisture content were recorded to account for pretreatment yield of solids (undissolved biomass).

Pretreatments for HLB were run using a full factorial experimental design with the following factors and levels: temperature (25, 35, 45, 55, and 65°C), time (1, 2, 4, 7, 8, and 12 weeks), and aeration (level 1 with air and level 2 without air), generating a total of 60 pretreated samples plus a replicate for each. All pretreatments for LLB were run with aeration and used a full factorial experimental design with the following factors and levels: temperature (55, 65, and 75°C) and time (1, 2, 3, 4, and 8 weeks) obtaining a total of 15 samples plus a replicate for each. To meaningfully compare HLB yields with LLB yields, HLB was also submitted to aerated pretreatments at 75°C for 1, 2, 4, and 8 weeks. Data for this higher temperature are shown in Table 1. After pretreatment, all samples were assessed for compositional analysis and enzymatic digestibility using the following laboratory analytical procedures.

Biomass composition

Analysis of raw and pretreated poplar wood was performed on samples with a particle size between 20 mesh (0.850 mm) and 80 mesh (0.180 mm), and a moisture content ≤10% as suggested by NREL Standard Analytical Procedures.[14] Extractives (i.e., chlorophyll, waxes, or similar organic components) were separated using 95% ethanol in an exhaustive extraction performed in a Soxhlet apparatus for 24 h. After extraction, the solvent was removed using a rotary evaporator (Buchi, Model 121), yielding the extracted compound that was quantified gravimetrically.[15] Carbohydrate, lignin, and acetic acid content were determined by submitting extractives-free samples to two-stage acid hydrolysis procedure.[13] The analyses for carbohydrates and acetic acid were performed on the resulting hydrolyzate by HPLC with refractive index detection using Biorad HPX-87P and HPX-87H columns, respectively. The lignin content was determined gravimetrically as the weight of solids after acid hydrolysis, discounting moisture and ash. Ashing was performed at 575 ± 25°C.[16]

Pretreatment liquor

The pretreatment liquor was separated from the solids through vacuum filtration. The concentrations of soluble monosaccharides and cellobiose were determined using HPLC equipped with Biorad HPX-87P and HPX-87H columns, and with refractive index detection. Whenever cellobiose was detected, the concentration was converted to glucose concentration using the conversion factor suggested in Section 8.20 of NREL Analytical Laboratory Procedure.[17] Oligomers were hydrolyzed before HPLC measurements by submitting the pretreatment liquor to acid hydrolysis (4% H_2SO_4) and then measuring dissolved sugars using HPLC with a Biorad HPX-87P column.[18]

Enzymatic hydrolysis

The sole criterion to determine recommended pretreatment conditions was the combined glucan and xylan yields after pretreatment and enzymatic hydrolysis. The cellulase (Spezyme CP®, lot 301-04075-054, activity 59 FPU/mL) used in this study was kindly provided by Genencor International. Its activity was monitored on a regular basis using NREL Standard Analytical Procedure.[19] A cellulase enzyme loading of 15 FPU/g glucan in raw biomass was used. β-glucosidase (Novozyme 188®, 288 CBU/g of activity as measured by Novo Nordisk Biochem) from Sigma-Aldrich was added with an excess loading of 60 CBU/g glucan in raw biomass.

The substrates used in this study were raw poplar wood and treated-neutralized-washed poplar wood. Based on moisture content, glucan content, and the solids pretreatment yield (dry weight pretreated biomass per weight of dry raw biomass), enough substrate was weighed to provide 0.1 g glucan for the reaction. Water, sodium citrate buffer (0.1 M, pH 4.8), antibiotics (tetracycline, 10 mg/mL in 70% ethanol and cycloheximine, 10 mg/mL in distilled water), and enzymes were added to the substrate to bring the total volume of the mixture to 10 mL.[20] After 72 h of hydrolysis at 50°C in a shaking incubator (Amerex Instruments, Laffayette, CA; 80 rpm), the sugar

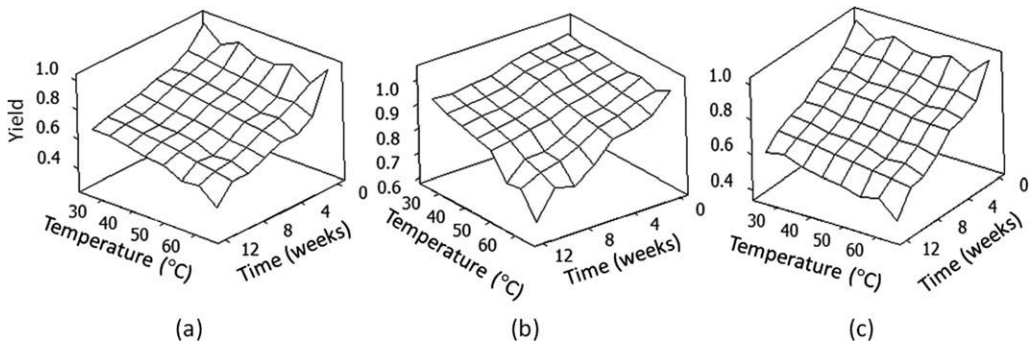

Figure 2. Effects of temperature and time on HLB pretreatment yield of (a) lignin, (b) glucan, and (c) xylan expressed as g of component removed per gram component in raw biomass.

yields were measured by HPLC using Biorad HPX-87P column with refractive index detection.

Data analysis method

Assessment of pretreatment yields of lignin, glucan, and xylan was based on the following definition for yield:

$$Y_i = \frac{C_i Y_T}{C_{i_0}} \tag{1}$$

where i = lignin L, glucan G, or xylan X, Y_i = pretreatment yield of Component i at time t (kg residual Component i/kg Component i in raw biomass), C_{i_0} = Component i content at time zero (kg Component i in raw biomass/kg raw biomass), C_i = Component i content at time t (kg residual Component i/kg residual biomass), and Y_T = total solids pretreatment yield at time t (kg residual biomass/kg raw biomass).

The highest possible yields are 1.0 and the amount of degraded component is $1.0 - Y_i$. The effects of pretreatment on lignin, glucan, and xylan yields for HLB are discussed in the next section and the differences with LLB are addressed in Section Comparing HLB and LLB pretreatment yields.

Overall yield (Y_{i_0}) is defined as the amount of glucan or xylan recovered after pretreatment and enzymatic hydrolysis per unit of cellulose or hemicellullose in the raw feedstock. Y_{i_0} and was calculated as:

$$Y_{oi} = Y_i \cdot Y_{ei} \tag{2}$$

where i = Glucan G or xylan X, Y_{oi} = overall yield of Component i (kg hydrolyzed Component i/kg Component i in raw biomass), Y_i = pretreatment yield of Component i (kg residual Component i/kg Component i in raw biomass), and Y_{ei} = enzymatic yield of Component i (kg hydrolyzed Component i/kg Component i in pretreated-neutralized-washed biomass).

To statistically determine significant differences of interest, Student t-tests and analysis of variance (ANOVA) were performed using Minitab® 15. Complete presentation of program outputs and check of statistical assumptions can be found elsewhere.[21]

Results and Discussion

Pretreated solids

Figure 2 shows that lignin, glucan, and xylan yields smoothly decreased with increasing time and temperature. Compared with lignin, the degradation extent was smaller for xylan and much smaller for glucan. According to Eq. 1, lignin, glucan, and xylan maximum yields were 1.0 and corresponded to untreated samples. The minimum yields were 0.29, 0.60, and 0.38 g component remaining per gram component in raw biomass, respectively, and corresponded to the aerated pretreatment at 65°C for 12 weeks. An ANOVA showed significant effect of control variables (time, temperature, and aeration) on lignin, glucan, and xylan yields (all P-values < 0.001); thus, different yields are expected for different pretreatment conditions. Nevertheless, the average lignin, glucan, and xylan yields were calculated for all 60 HBL samples. These data are important to this study because they indicate overall performance of lime pretreatment on wood. These average yields were 0.76, 0.92, and 0.78 g component remaining per gram component in raw biomass, respectively. The average glucan yield is very high showing good cellulose preservation. On the other hand, typical lignin yields were much lower showing that lime pretreatment selectively removes lignin. Covalent bonds between hemicellulose and lignin have been demonstrated for grasses[22] and may be the case for other herbaceous biomass[23,24] but this type of bonding has not been proved for woody biomass (a good discussion can be found in the review by Helm[25]). In any case, lignin and hemicellulose are closely associated with each other,

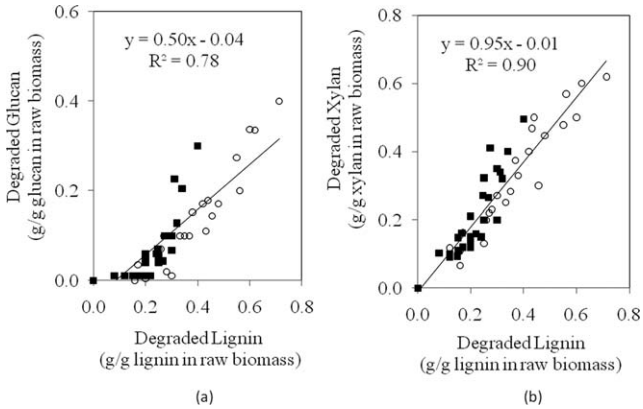

Figure 3. Degraded (a) glucan and (b) xylan compared to degraded lignin for HLB pretreatment. ○ aerated; ■ nonaerated.

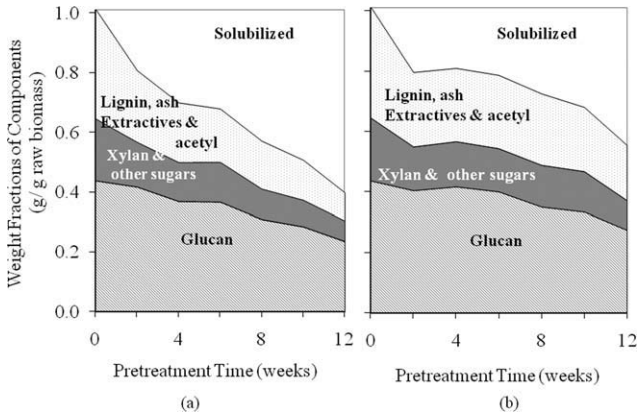

Figure 4. Mass profiles of raw and treated HLB poplar wood with aeration at (a) 65°C and (b) 25°C.

which would explain why lignin and hemicellulose yields are similar.

Figure 3 shows degraded glucan ($1.0 - Y_G$) and degraded xylan ($1.0 - Y_X$) compared against degraded lignin ($1.0 - Y_L$). Glucan and lignin degradations are weakly related (lower slope and lower coefficient of determination), whereas xylan and lignin degradations are strongly related (higher slope and higher coefficient of determination) indicating that lignin is structurally more related to xylan than to glucan. More insights on these phenomena are obtained through kinetic modeling of lime pretreatment, which allows estimation of lignin, glucan, and xylan yields, and also calculation of selectivity as a function of pretreatment conditions (Sierra et al., unpublished data).

Aerated and nonaerated modes were compared by calculating average differences in yields using a two-sample *t*-test for each of the three components: lignin, glucan, and xylan (the hypotheses were for differences in average yields in the nonaerated mode minus average yields in the aerated mode). The results for lignin, glucan, and xylan with the corresponding confidence intervals were 0.12 ± 0.081, 0.055 ± 0.048, and 0.082 ± 0.087 g remaining component per gram component in raw biomass, respectively. In the same order, the *P*-values were 0.005, 0.027, and 0.120. These results indicate that lignin and glucan yields in the aerated mode are significantly different and smaller than the corresponding yields for nonaerated pretreatment (within 5% significance level). For xylan, the high *P*-value shows that there may be an effect due to the presence of air, but it is less notable. This suggests that mechanisms for xylan degradation are not as strongly influenced by oxygen; but it is more likely alkaline-catalyzed hydrolysis than radical attack, unlike lignin degradation.

Regarding other components, total removal of acetyl and partial (50%) removal of extractives were observed within the first week of pretreatment and remained almost constant until the 12th week of pretreatment. Complete mass profiles were obtained for all components at all pretreatment conditions, but the only results presented here are for aerated pretreatments at 65 and 25°C (Figure 4). For both aerated and nonaerated pretreatments, the general tendency is for rapid solubilization of components during the first 1 to 2 weeks. Later, solubilization rates were almost constant and generally higher for the aerated mode.

These results show that lime pretreatment significantly changes biomass composition, with the strongest effect on lignin removal.

Comparing HLB and LLB pretreatment yields

Figure 5 compares HLB yields against LLB yields for oxidative pretreatment at 65°C. The significance of observed differences was assessed through an ANOVA, which showed that temperature, time, and batch have significant effects on lignin and glucan yields at $\alpha = 3\%$; however, xylan yield is not affected by temperature, only by time and batch (the *P*-values were 0.643 for temperature and <0.012 for time and batch). Factors that may be responsible for the differences between the two batches of poplar wood are (1) diverse composition or distribution of lignin and hemicellulose, (2) distinct spatial configurations, (3) dissimilar bonding between lignin units, (4) different bonding between lignin and carbohydrates, and (5) important differences in the initial amount of lignin and carbohydrates for the two batches of biomass. Unfortunately, more specificity regarding possible differences is out of the scope of this article, but it is clear that for HLB, xylan, and lignin degradation were higher, whereas glucan preserved better.

According to the above referenced ANOVA, xylan yield is independent of temperature. This result is opposite to the result shown in the previous section for a wider range of temperatures (25–65°C), implying that temperature has an effect on xylan degradation, but the range from 55 to 75°C is too small to be significant. Furthermore, xylan yield was independent of aeration (as discussed in the previous section); thus, unlike lignin and glucan degradation, xylan degradation is not strongly influenced by reaction conditions (temperature and aeration) provided temperature >55°C. Instead, xylan degradation depends primarily on time.

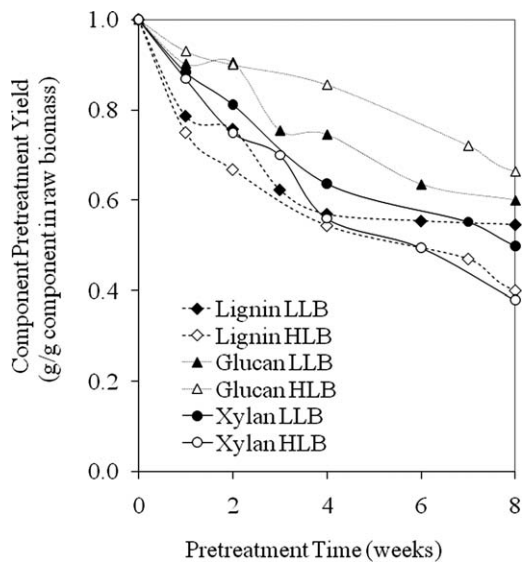

Figure 5. Comparative effects of aerated pretreatment at 65°C on different batches of poplar wood.

Table 2. Percentage Decrease in Oxygen Partial Pressure in Air Saturated with Water at Temperature T (°C) Compared with 25°C*

Temperature (°C)	Saturation pressure (kPa)	Vapor pressure (kPa)	Oxygen (molar fraction)	Decrease in oxygen concentration (%)
25	3.17	1.91	0.203	0.0
50	12.4	7.41	0.184	9.35
55	15.8	9.45	0.177	12.8
57	17.9	10.7	0.173	15.0
60	19.9	12.0	0.169	17.1
65	25.0	15.0	0.158	22.3
70	31.2	18.7	0.145	28.5
75	38.6	23.1	0.130	36.1
80	47.4	28.4	0.112	45.1
85	57.8	34.7	0.090	55.7
90	70.1	42.1	0.065	68.2

*Saturated air (100% humidity) and standard atmospheric pressure 101,325 Pa.

In this comparative study, temperature was increased to 75°C. Inherent delignification rates are triggered by higher temperatures, that is, rate constants are higher (Sierra et al., unpublished data). However, because long-term lime pretreatment uses 1-atm total pressure, higher temperatures increase water vapor pressure, and reduce oxygen partial pressure. Table 2 shows that at 75°C, oxygen partial pressure reduces by 36% compared to the partial pressure at 25°C.[26] According to the statistical analysis, a temperature of 75°C significantly affects lignin degradation; however, it also degrades carbohydrates more rapidly. The total effect of this temperature can only be evaluated by calculating overall pretreatment and enzymatic hydrolysis yields (Eq. 2) as discussed in Section Enzymatic and Overall Hydrolysis Yields.

Pretreatment liquor

Sugars in the pretreatment liquor were <0.010 g sugar recovered per gram sugar in raw biomass. Glucan oligomers were 0.010 g glucan recovered per gram raw biomass, and xylan oligomers were 0.013 g xylan recovered per gram raw biomass. Even though degradation products from lignin and carbohydrates reactions triggered in the alkaline media are present in the pretreatment liquor, these were not quantified

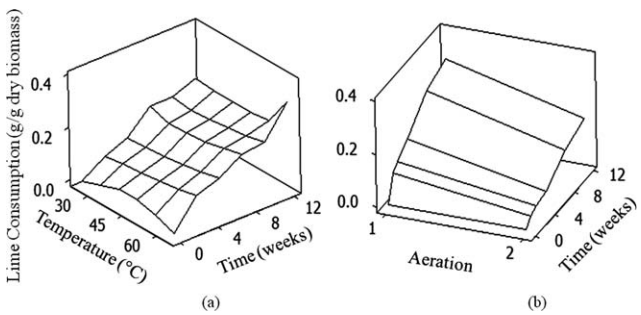

Figure 6. Surface plots to assess the effects of (a) temperature and time averaged over aeration, and (b) aeration and time averaged over temperature on lime consumption during pretreatment of HLB.

Lime consumption is expressed as g $Ca(OH)_2$ consumed per gram dry biomass.

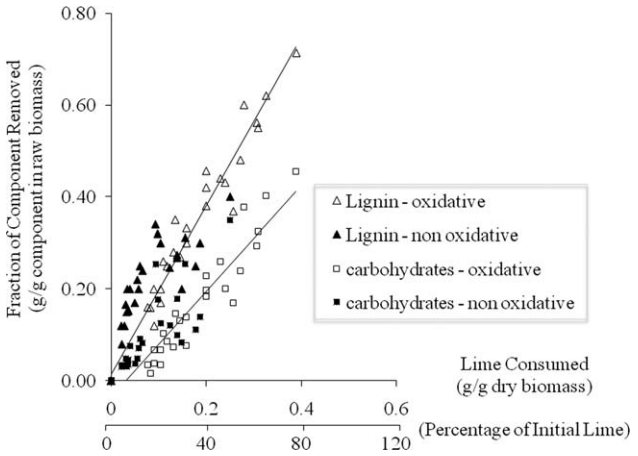

Figure 7. Component removed as a function of lime consumed for HLB pretreatment.

because previous studies showed that lime pretreatment degradation products do not inhibit fermentation.[27]

Lime consumption

Longer pretreatments, higher temperatures, and aeration cause higher lime consumption (Figure 6). These effects were statistically ascertained through an ANOVA that gave P-values of 0.018 for temperature, and <0.001 for both time and aeration. Thus, the effects of temperature, time, and aeration on lime consumption are statistically significant at $\alpha = 2\%$.

The presence of oxygen provides new pathways to degrade lignin, including a dominant phenolic delignification (oxidation of the phenolic subunits of lignin rather than the nonphenolic). In other words, unlike the nonoxidative alkaline process, oxidation attacks C—C bonds.[28] Average lime consumption was 0.11 g $Ca(OH)_2$/g dry biomass. The maximum was 0.33 g $Ca(OH)_2$/g dry biomass and was observed for 12-week oxidative pretreatment at 65°C. Lime consumption was 0.20 g $Ca(OH)_2$/g dry biomass (or 40% of initially loaded lime) for the recommended conditions of aerated pretreatment at 65°C for 4 weeks (see Section Enzymatic and Overall Hydrolysis Yields).

The relationship between lime consumption and lignin or carbohydrate removal is linear (Figure 7), particularly for lignin with aeration. This linearity means that lime is stoichiometrically consumed in the degradation reactions. Table 3 shows parameters for linear regression models for both lignin and carbohydrates in the aerated and nonaerated modes, with their statistical indicators. Compared with the nonaerated counterpart, the aerated mode shows better fit and higher slopes for both lignin and carbohydrates. Consequently, in the presence of air, lignin and carbohydrate degradation are greater per unit of consumed lime. In other words, the aerated mode consumes less lime to obtain a desired delignification level.

Enzymatic and overall hydrolysis yields

This study was designed to assess pretreatment not to optimize enzymatic hydrolysis variables; thus, fixed and favorable enzymatic hydrolysis conditions were used (see Section Enzymatic Hydrolysis). Hemicellulose hydrolysis was not

Table 3. Regression Parameters and Statistical Indicators for a Linear Regression Model Relating Lime Consumption to Lignin or Carbohydrates Degradation

Regression Model	Intercept		Slope		R^2	Regression *P*-value
	a	*P*-value	*b*	*p*-value		
LD* = $a \times$ LC† + *b* aerated	1.83	<0.001	0.015	0.279	95.8	<0.001
CD‡ = $a \times$ LC† + *b* aerated	1.14	<0.001	–0.035	0.015	89.8	<0.001
LD* = $a \times$ LC† + *b* nonaerated	1.40	<0.001	0.085	<0.001	67.5	<0.001
CD‡ = $a \times$ LC† + *b* nonaerated	1.08	<0.001	0.0075	0.572	68.6	<0.001

*Lignin degraded.
†Lime consumed.
‡Carbohydrates degraded.

specifically addressed by the addition of xylanases to the enzymes cocktail. Spezyme CP®, which is a *Trichoderma reesei* cellulase enzyme complex, includes some xylanase activity; thus, hemicellulose hydrolysis under the conditions used in this study has been observed by others.[29]

To select the best pretreatment conditions, the sole criterion was the overall combined glucan and xylan yield, which is discussed next for HLB. (Note: Results for LLB are presented in Section Comparing HLB and LLB Overall Yields).

Figure 8a shows that glucan and xylan digestibleness of pretreated biomass are closely correlated; as xylan digestibility improves, so does glucan digestibility.[4] Interestingly, Figure 8b shows that as lignin is removed, there is strong preference for glucan hydrolysis over xylan hydrolysis. This result can be explained by the fact that as lignin is removed, hemicellulose is preferentially removed (see Figure 3). As hemicellulose is removed, it opens pores giving greater access to cellulose, similar to what is observed in dilute acid pretreatment.[30]

Furthermore, Figure 8c shows that at 65°C, there is a preference for glucan digestion if the biomass was pretreated with aeration (i.e., ratio is generally >1.0), whereas there is a preference for xylan digestion if the biomass was pretreated without aeration (i.e., ratio is < 1.0). As pretreatment proceeds, both aerated and nonaerated modes show increasing preference for glucan (i.e., the slopes for both lines are positive). More lignin and hemicellulose removal occurs at longer pretreatments, so there is greater enzymatic access to cellulose, which increases its digestibility.

The maximum glucan digestibility was obtained in the aerated mode for the 65°C pretreatment that lasted 4 weeks. Under these conditions, glucan yield was 0.95 g/g glucan in treated biomass with a corresponding xylan digestibility close to 0.80 g/g xylan in treated biomass. In the nonaerated mode, the maximum glucan and xylan digestibleness were both close to 0.60 g/g carbohydrate in treated biomass.

Figure 9 shows the effect of pretreatment variables on combined glucan and xylan overall yields. Higher temperatures and aeration improve yields showing a slight constant positive slope. Conversely, pretreatment times lower than 2 weeks gave very low yields. Using ANOVA, the effect of temperature, time, and aeration on the overall combined yield was corroborated with *P*-values <0.030.

A contour plot (Figure 10) shows that the most promising overall yields are approximately constant for times >2 weeks and temperatures ≥55°C. For these conditions, average overall combined yields were 0.52 and 0.39 g glucan + xylan recovered per gram glucan + xylan in raw biomass for the aerated and nonaerated pretreatments, respectively. Considering the combined overall yield of raw poplar (0.10 g glucan + xylan recovered per gram glucan + xylan in raw biomass), this result indicates that aerated lime pretreatment consistently increases the digestibility of raw poplar wood by an average factor of about 5 with respect to raw biomass. The highest improvement in digestibility was about 7.5-fold (0.76 g glucan + xylan recovered per gram glucan + xylan in raw biomass) obtained for aerated pretreatment at 65°C

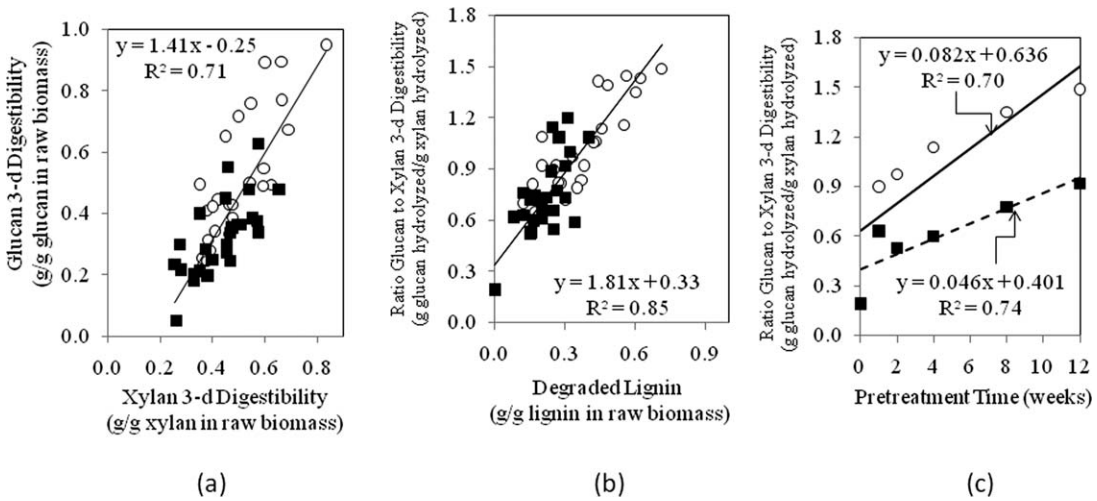

Figure 8. (a) Glucan digestibility compared with xylan digestibility. Ratio of glucan to xylan compared with (b) degraded lignin, (c) 65°C pretreatment time. ○ aerated; pretreatment ■ nonaerated pretreatment.

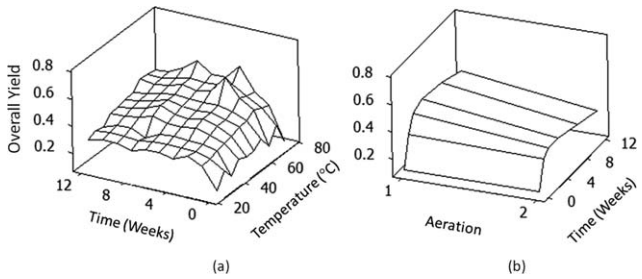

Figure 9. Surface plots to assess the effects of (a) temperature and time averaged over aeration and (b) time and aeration averaged over temperature on overall yield of combined glucan and xylan of HLB expressed as g glucan + xylan recovered per gram glucan + xylan in raw biomass.

and 4 weeks; consequently, these are the recommended pretreatment conditions for poplar wood.

More severe oxidative lime pretreatment conditions (oxygen pressure up to 28 bars and temperature up to 180°C) result in a much higher poplar wood digestibility as discussed elsewhere.[12]

Comparing HLB and LLB overall yields

Differences in combined overall yield for HLB and LLB are negligible (Figure 11). A mean difference (LLB yields minus HLB yields) of 0.023 ± 0.14 was observed; thus, the effect of batch on overall yield is not significant. As discussed in Section Comparing HLB and LLB pretreatment yields, pretreatment yields of lignin, glucan, and xylan for HLB were different from those of LLB. In particular, lignin degradation was more extensive for LLB; however, LLB was as digestible as HLB. This phenomenon is explained because only some delignification (50% according to Zhu et al.[31]) is required to remove the hindrance of lignin to enzyme attack; further delignification is not necessary. Besides, xylan degradation was more extensive and glucan was better preserved in the pretreatment for LLB than in the case of HLB. As a consequence of all of these pretreatment

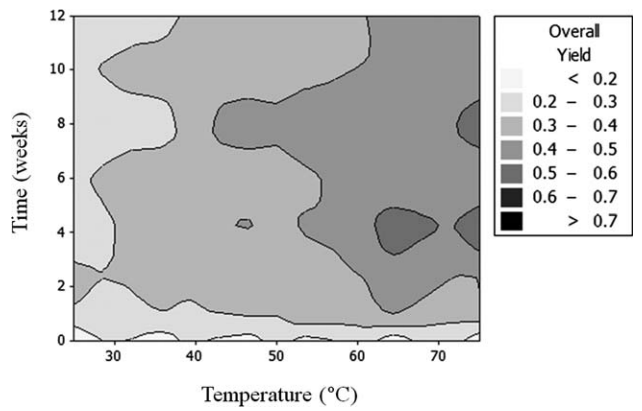

Figure 10. Contour plot for overall yield of HLB as a function of time and temperature.

Overall yield is expressed as g glucan + xylan recovered per gram glucan + xylan in raw biomass.

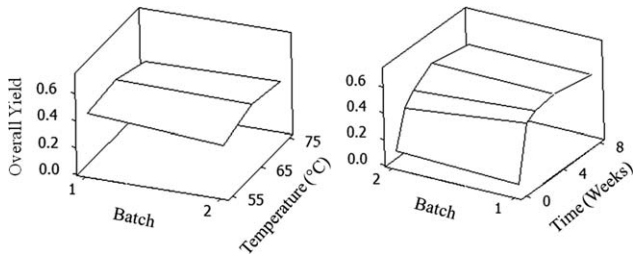

Figure 11. Effects of temperature, batch, and time on overall yield of combined glucan and xylan expressed as g glucan + xylan recovered per gram glucan + xylan in raw biomass.

and enzymatic yields, overall combined glucan + xylan yields were comparable for both batches.

In another statistical analysis, the effects of time and temperature were separately tested from the effect of batch on combined overall yield. An ANOVA showed the following *P*-values for a test on the effects of time, temperature, and batch: <0.001, 0.056, and 0.331, respectively. Consequently, although the effects of time and temperature are significant within $\alpha = 6\%$, the effects of batch are unimportant. With these results, the discussion for HLB in Section Enzymatic and Overall Hydrolysis Yields can be extended to LLB.

Regarding temperature, 75°C gave higher delignification but because sugar degradation was also greater, the overall result is that 65°C gives better overall yields.

Conclusions

Long-term lime pretreatment produces significant changes in poplar wood composition, mainly reducing lignin and hemicellulose. The extent of change is a function of time, temperature, and aeration. Xylan pretreatment yield is strongly influenced by time, whereas temperature and aeration have a much smaller effect.

Lime consumption is linearly related to lignin and carbohydrate degradation with better fit and greater slopes for the aerated mode. To achieve a given delignification, less lime is required in the aerated mode than in the nonaerated mode.

Biomass pretreated with aeration for more than 2 weeks at 65°C and submitted to subsequent enzymatic hydrolysis with 15 FPU/g glucan in raw biomass showed an average improvement of approximately five-fold in digestibility. For aerated pretreatment at 65°C and 4 weeks, the improvement was 7.5-fold (0.76 g carbohydrates recovered per gram carbohydrates in raw biomass).

Differences in raw poplar lignin content (21.4 and 29.1%) for different batches of the feedstock gave different pretreatment yields, but overall yields were comparable.

Acknowledgment

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